Production of Shelf-Stable Hydrogel Microparticles

using 25% kappa-carrageenan with 75% alginate for encapsulation of anthocyanins

A Thesis

Presented in fulfillment of the Requirements for Graduation with Honors Research Distinction in the College of Engineering at

The Ohio State University

By

Amanda Broseus

The Ohio State University

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Project Advisor: Gönül Kaletunç

Abstract

The research results in the area of improving human health and wellness are in high demand for the food industry. Human beings are becoming more focused on healthy living through consumption of healthy foods and beneficial compounds. Beneficial compounds tend to be sensitive to environmental conditions such as pH, light, and oxygen. Anthocyanins (ACN) have antioxidant and anticancer properties as well as provide natural color to the food product. However, due to their instability during processing and storage, their encapsulation is necessary to improve stability and targeted delivery in the human body. Selection of encapsulation material is critical for protection of ACN and for its optimum delivery.

Kappa-carrageenan, extracted from red seed weed and alginate, extracted from brown algae were used to form hydrogel for encapsulation of ACN extracted from purple corn and blueberry. Kappa-carrageenan forms hydrogel with potassium chloride (KCl) while alginate forms with calcium chloride ($CaCl_2$). Hydrogels were formed by adding a blend of kappa-carrageenan and alginate into water at 70 ^oC. After dissolution, the mixture was cooled down to 40 ^oC and ACN was added. The resulting solution was dripped with a peristaltic pump into $CaCl_2$ + KCl added 0.1M pH 3 buffer to form spherical hydrogel particles. Desorption isotherms of hydrogels were developed to determine the drying parameters of hydrogels. Hydrogel particles in fully hydrated and dried forms were placed in a buffer solution and the diffusion of ACN from hydrogels was characterized by using a spectrophotometer or a fluorometer, to determine the effects of drying on the release rate of ACN from the hydrogels.

Results indicate that encapsulation efficiency in hydrogels is affected by the botanical origin of ACN as higher encapsulation efficiency was observed for blueberry ACN than for

purple corn ACN. The wet and dry particles diffusion results showed that the leakage from hydrogel particles can be reduced by drying.

Acknowledgments

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Introduction

The food industry is highly interested in process of encapsulation for targeted delivery of beneficial compounds to the human body. An encapsulated particle is composed of two parts, an outer shell and the core it protects from interaction with environmental factors such as temperature or pH level changes, oxygen exposure, and other ingredients or components in the system. Hydrogels of synthetic polymer or biopolymer based are often used in forming particles of various sizes (nanometer to millimeter scale) (Higuita-Castro, et al, 2012). Elasticity and strength of gels are important to evaluate the stability of particles during processing and storage. Elasticity of a gel is measured by using successive compression-decompression cycles to determine the degree to which the gel returns to its original shape, while the strength of a gel is determined by compressing the gel to the failure point (Kaletunc et al., 1991).

Alginate is the most widely used shell material for encapsulation and will form gels in the presence of calcium chloride. Kappa-carrageenan has also high potential to be used as encapsulate material because of its thermal reversibility and its gelling ability in the presence of potassium chloride. As the level of potassium is increased, the gel structure becomes tightly aggregated and gel strength increases (Marine Colloids Carrageenan). Pharmaceutical industry utilizes synthetic polymers such as Eudragit L or Eudragit S (based on methacrylic acic and methyl methacrylate) for colon specific drug release due to their response to pH stimuli (Higuita-Castro, et al, 2012). However, FDA requires use of food approved polymers for food applications. A hydrogel prepared using a blend of alginate and pectin was reported to stay intact at low pH but dissolve at basic pH in human intestines (Guo and Kaletunc, 2016). Because the kappa carrageenan gels also have desirable gel properties of high strength and elasticity, it is

essential to explore the possibility of blending kappa-carrageenan with other polymers to prepare hydrogels responsive to pH stimuli.

The hydrogels are expected to be manufactured by an ingredient company and purchased by food production companies to be incorporated into foods. This approach will work best if the hydrogels are prepared to be shelf-stable; which can be accomplished by drying to a water activity of 0.4 or lower. Drying studies were performed in an oven to determine the drying time to reach a water activity of less than 0.4. Previous work in our laboratory had indicated that complete drying hydrogels to almost zero moisture content causes disintegration of hydrogel structure upon rehydration. For hydrogel characterization at multiple moisture contents desorption isotherms were constructed.

For application in the food industry other properties, including color and nutrition, are important in addition to the structural properties of the hydrogel. Along with the health benefits of cancer, diabetes, cardiovascular illness prevention, and neuronal diseases prevention (Yousuf et. al, 2016), anthocyanins also offer attractive colors. Anthocyanins from purple corn and from blueberries, encapsulated inside hydrogels, may offer a purple color because the kappa-carrageenan- alginate hydrogel is transparent. Since color is one of the first characteristics the human eye notices when evaluating a food (Barry, 2013) these colors may make the encapsulations more aesthetically pleasing; as long as they maintain the color and it does not diffuse out of the hydrogel particles before consumption. The absorbance of the curing solution was determined with a spectrophotometer at a wavelength of 520nm after curing was completed to determine the encapsulation efficiency. The diffusion of the anthocyanins from wet or dry hydrogels were tested through fluorescent monitoring by placing hydrogels in pH 3 buffer.

Materials and Methods

Materials

Gelcarin GP-911 NF, kappa-carrageenan (kC), was provided by FMC Biopolymer (Philadelphia,PA), Potassium Chloride (KCl) was purchased from Fisher Scientific. Purple corn extract (ACN) was provided by Dr. Giusti's Lab (The Ohio State University) in powder form. Blueberry (ACN) was supplied by SVZ (Othello, WA) in a juice concentrate. Alginate (Protanol SF120RB) was provided by FMC Biopolymer (Philadelphia, PA). Calcium Chloride was purchased from Fisher Scientific.

ACN Solution Preparation

The purple corn extract was added to pH 3.0 buffer 3 grams of powder with 13 grams of buffer to prepare ACN solution to be used in formulation. The blueberry extract solution was diluted by mixing with pH 3.0 buffer (3 grams of extract with 6 grams of buffer) to prepare the ACN solution to be used for gel formulation.

Alginate:Kappa-Carrageenan Gel Solution Preparation:

Water was heated to 70 ^{o}C and a blend of kC (25% wt) and alginate (75% wt) was slowly added to the heated water. The solution was mixed until the ingredients were fully dissolved. ACN solution was slowly added after the mixture cooled to 40 ^{o}C. Water evaporated during heating process was added back to reach to the desired concentration.

Alginate:Kappa-Carrageenan Hydrogel Particle Production

Using a peristaltic pump (Cole Parmer, IL) the kC-aginate-ACN solutions were extruded through a 0.337 mm inside diameter (23G) needle (Hamilton, Nevada). 10.2 g of the droplets were extruded at a volumetric flowrate of 13 mL/min into 75g of a 0.1 M pH 3.0 citrate buffer with added $CaCl_2$ and KCl (1.054g $CaCl_2$ + 0.708g KCl per 95.0g buffer) as shown in Figure 1.

Particle weight to buffer solution weight ratio was kept constant at 0.136. Upon contact with the gently agitated buffer the solution droplets formed hydrogel particles. The hydrogel particles were cured in the buffer solution in a dark space for 2 hours at 4 °C.

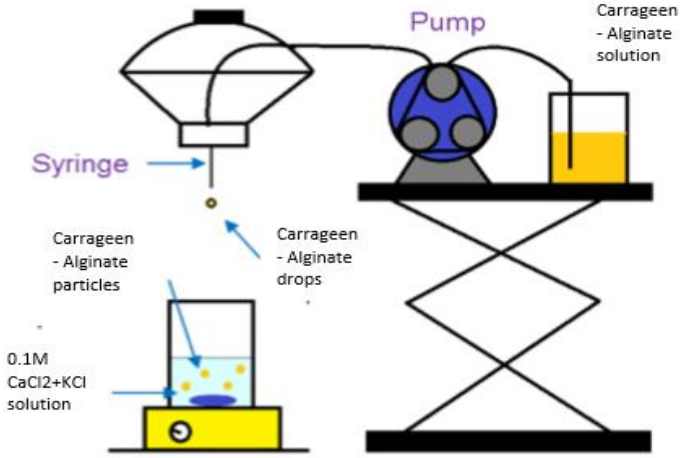

Figure 1: Hydrogel particle production

After the curing time the hydrogels were removed from the curing solution using a filtration funnel with vacuum depicted in Figure 2.

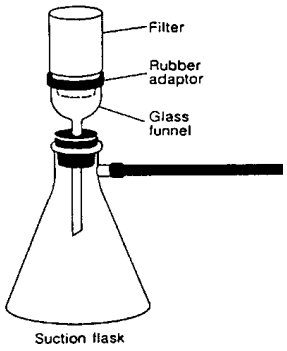

Figure 2: Filtration funnel with vacuum for particle separation

Encapsulation Efficiency of Hydrogels

Encapsulation efficiencies for the hydrogels were determined with Equation 1:

$$EE\ (\%) = \frac{initial\ ACN\ in\ dropped\ gel - final\ ACN\ in\ curing\ bath}{initial\ ACN\ in\ dropped\ gel} * 100 \qquad \text{Equation 1}$$

Desorption Studies of Hydrogel Particles in a Desiccator

Two separate studies with purple corn ACN were performed within desiccators containing various saturated salt solutions to equilibrate hydrogels in various relative humidity environments over a period of seven days after curing of particles. The particles after curing were placed in trays and then into one of ten separate glass desiccators (Figure 3). Saturated salt solutions were used to obtain desired relative humidity levels inside the desiccators. A third desiccator study was performed with purple corn ACN, but only with the KOH, LiCl, KCH_3COO-, and MgCl salt solutions containing desiccators. This third test was done because the moisture content after equilibration of the particles in relatively low humidity environments were higher than expected.

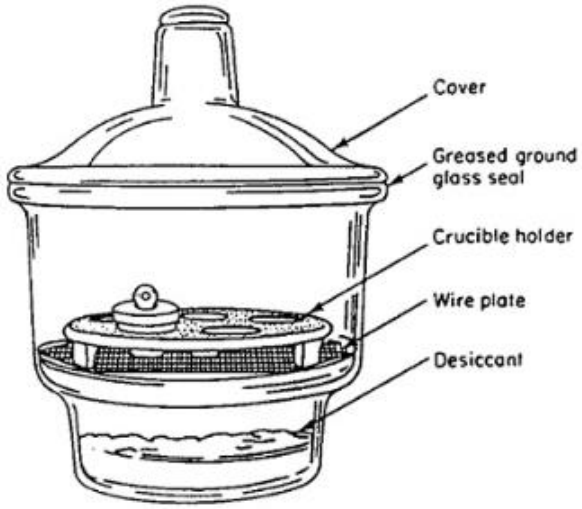

Figure 3: Desiccator (Apparatus and Experimental Techniques, 2009)

Saturated salt solutions and corresponding water activities are shown in Table 1.

Table 1: Salts used to create various relative humidity conditions

Salt	a_w	Salt	a_w
KOH	0.0824	$Mg(NO_3)_2$	0.529
LiCl	0.11	NaCl	0.75
KCH_3COO-	0.23	KCl	0.85
$MgCl_2$	0.33	KNO_3	0.94
K_2CO_3	0.43	K_2SO_4	0.973

Drying of Hydrogel Particles in an Oven

Hydrogel particles were placed on trays inside a preheated oven at 50 °C for drying. The trays were removed at various time intervals and the dried particles were placed into a water activity measurement unit (Novasina, AW Sprint, Switzerland) to determine the drying time to reach a water activity level of just below 0.4 to prevent mold growth and to produce shelf stable hydrogels for transportation and storage.

Diffusion of Anthocyanins from Hydrogels

ACN diffusion from both wet and dry hydrogels was determined with fluorometer measurements. The wet hydrogels used for testing were used directly after removal from the curing bath; the dry hydrogels used were used directly after oven drying. 0.5 grams of hydrogels (initial wet weight) were mixed with 2.0 mL of pH 3.0 citrate buffer inside of a cuvette and was placed in the fluorometer. An intensity measurement was taken at an emissions wavelength of 380 nm as a function of time.

Data Analysis

The moisture contents were calculated using the following equation, Equation 2:

$$MC = \frac{Initial\ Weight - Weight\ after\ drying}{Weight\ after\ Drying} * 100\% \qquad \text{Equation 2}$$

Results

Desorption studies of hydrogel particles:

The equilibrium moisture contents of hydrogel under various relative humidity conditions were determined by placing them inside desiccators over saturated salt solutions. The equilibrium moisture contents are plotted against water activity (a_w) for purple corn ACN containing hydrogel particles (Figure 4). The final equilibrium moisture contents of particles are given in table A1 in the appendix.

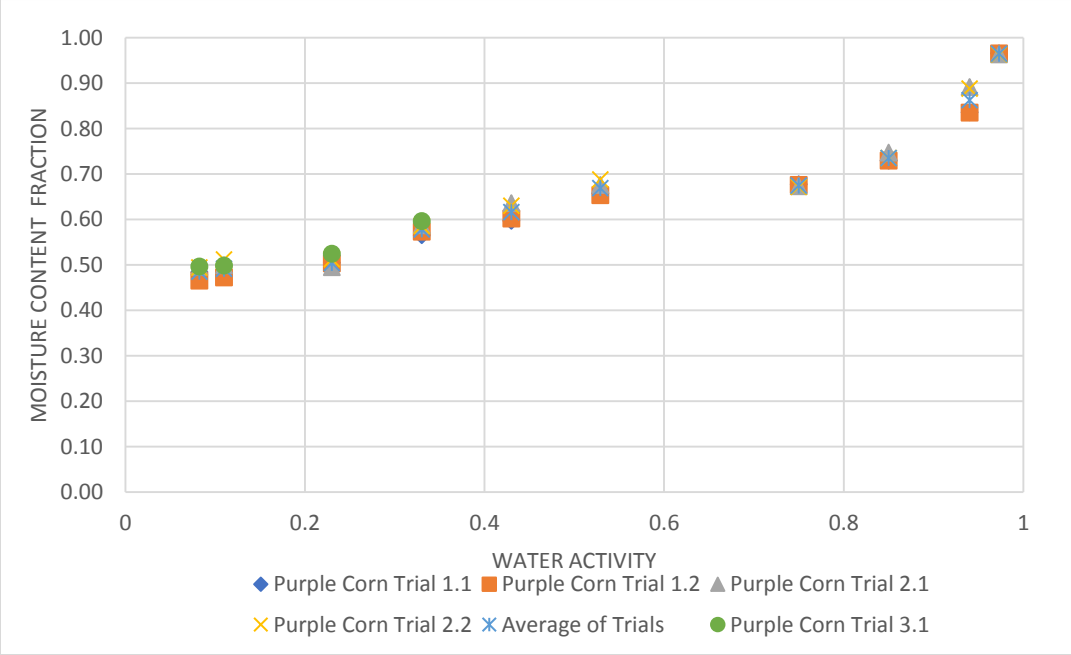

Figure 4: Desorption isotherm of hydrogels with purple corn ACN

The similar desorption studies conducted in duplicate were performed with hydrogel particles containing blueberry ACN. Figure 5 shows the individual studies and average equilibrium moisture contents of the hydrogel particles in each desiccator. The data are provided in table A2 in the appendix.

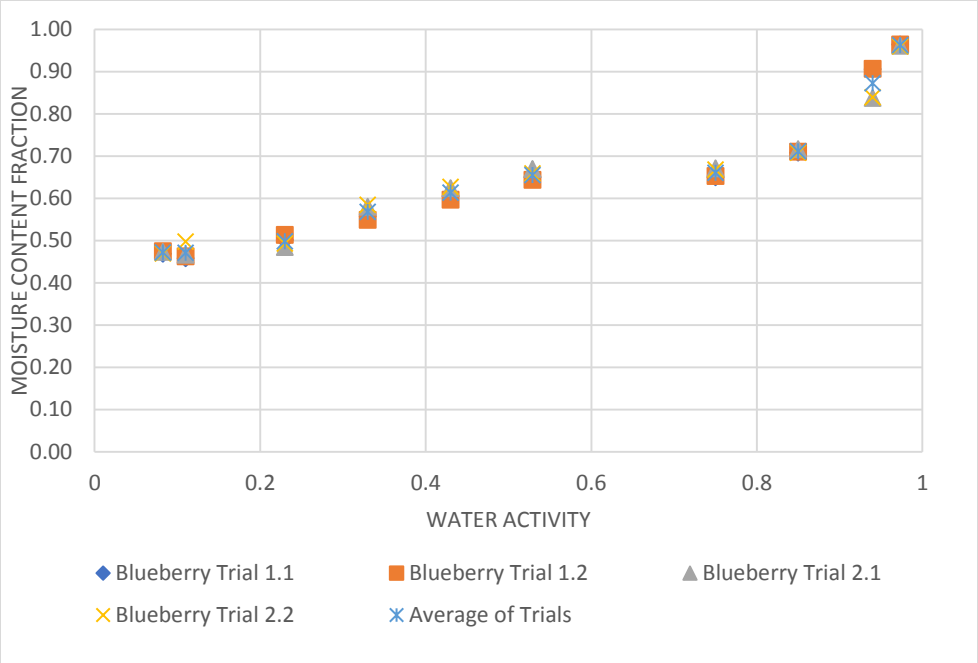

Figure 5: Desorption isotherm of hydrogels with blueberry ACN

The encapsulation efficiencies were calculated based on the absorbance measurements of the anthocyanin solution in the curing bath and applying a mass balance for ACN (Table 3). The data collected for calculation of encapsulation efficiencies are given in table A3 in the appendix. For the purple corn ACN, trial 1 had higher encapsulation efficiency than trials 2 and 3 which are very similar. Trial 1 had a higher initial anthocyanin content than trials 2 and 3. While the initial anthocyanin content of blueberry trials were lower than those of purple corn, encapsulation efficiency of blueberry ACN was higher.

Table 3: Encapsulation efficiencies

	Purple Corn Trial 1 Data	Purple Corn Trial 2 Data	Purple Corn Trial 3 Data	Blueberry Trial 1 Data	Blueberry Trial 2 Data
Anthocyanin concentration in gel solution (ug/ml)	160.98	111.70	111.77	9.29	9.29
Encapsulation efficiency (%)	23.3	12.6	13.2	37.0	36.5
Average EE	23.3	12.9		36.7	

Modeling of desorption isotherms:

GAB (Guggenheim-Anderson-de Boer) equation (Equation 3) was selected to fit the desorption data for hydrogel by using the Kaleidagraph software.

$$m = \frac{(Ckm_o a_w)}{(1-ka_w)(1-ka_w+Cka_w)} \qquad \text{Equation 3}$$

where; m=moisture content a_w=water activity C, k, m_o are = constants determined through Kaleidagraph curve fit option.

The plots Figure 6 and 7 are the Water Activities vs Moisture Content. The initial guesses for these values were based on GAB Parameters for corn (Bell and Labuza, 2000).

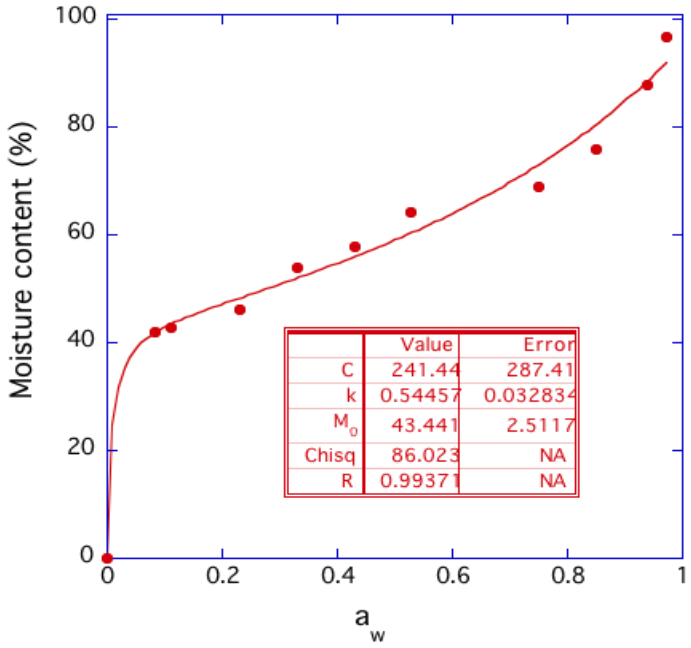

Figure 6: Purple corn ACN desorption isotherm

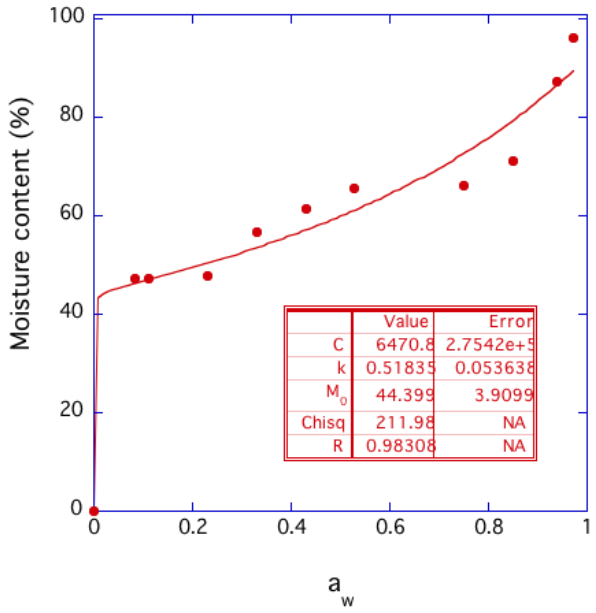

Figure 7: Blueberry ACN desorption isotherm

Oven drying studies:

The oven drying study was conducted at constant temperature over a time frame of 5.5 hours with a preheated oven at 50 °C. Four trays of particles were placed into the oven at the same time and at each time interval a tray was removed and immediately placed inside the water activity measurement equipment. Water activity measurements are performed at 24.9 °C. (Table 3). All drying times tested were sufficient in lowering the moisture content to below the shelf stable value of 0.4, indicating that the shortest time frame of 2 hours would be sufficient.

Table 3: Oven drying test data

Oven Test Data		
Time in Oven (hr)	a_w Reading	Temperature of Analyzer (◦C)
2	0.368	24.9
3.5	0.368	24.9
4	0.351	24.9
5.5	0.347	24.9

Diffusion studies of wet and dry particles:

The diffusion of ACN, from hydrogel particles with the purple corn ACN, was monitored over time with a fluorometer in terms of fluorescence intensity as a function of time. For the wet and dry particles, the intensity vs time data are shown in Figure 8. The intensity values of emission were measured at 380nm. The intensity increases as a function of time and reaches to an asymptotic value when an equilibrium is established between the anthocyanin concentration in solution and inside the particles. Comparing the intensities at equilibrium of the wet versus dry particles showed that the wet particles in buffer had a higher intensity of around 2200, whereas the dry particles in buffer solution had an intensity of just 800. This indicates that a larger amount of ACN diffuses out of the wet particles than dried particles.

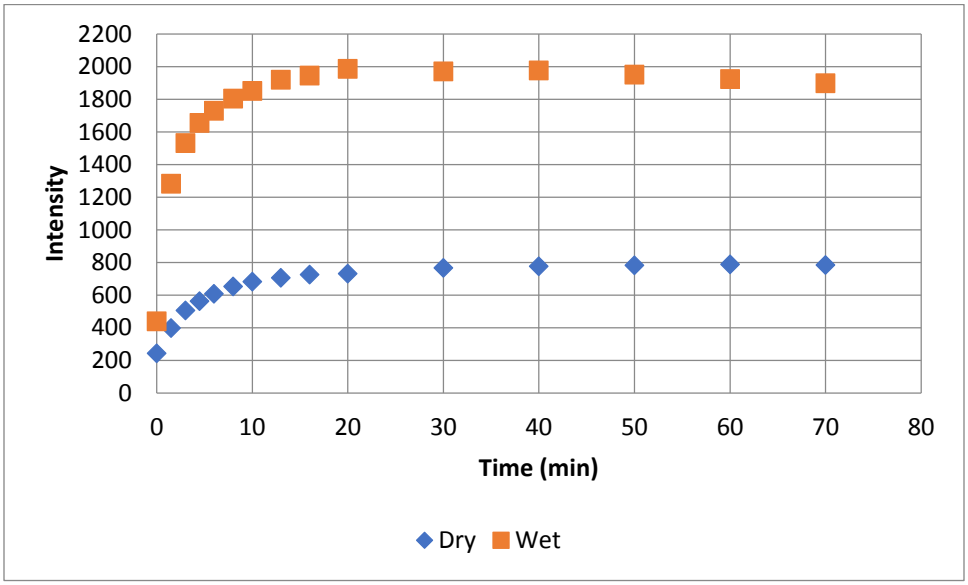

Figure 8: Diffusion of purple corn ACN from wet and dry particles

Discussion

This project investigated the use of kC and alginate, in a 25:75 weight ratio to produce hydrogels to encapsulate purple corn and blueberry anthocyanins. These hydrogels were intended to be used as ACN carrying vehicles in the food and beverage industries. ACNs require encapsulation because of their instability when they are exposed to high temperature, oxygen and light, as well as during processing and storage (Robert et. al, 2015). ACNs are also affected by pH and are generally only stable at pH values at or below 3.5 (Robert et. al, 2015). ACN containing hydrogels should have a minimum release of ACN before the targeted delivery area is reached. For an application, such as incorporation into a liquid product, the stability of the shell materials (alginate and kC in our case) are important. Therefore, the diffusion of ACN from the hydrogels during curing and after drying were investigated. Oven drying parameters were also evaluated with the intention of lowering hydrogels moisture content to create a shelf-stable product.

Hydrogels formed from alginate using $CaCl_2$ have been used throughout various industries including encapsulation of proteins and drugs. Alginate hydrogels are known to be stable at high temperatures (Guo and Kaletunc, 2016). The mechanical strength of hydrogels increase with increasing alginate concentration which is attributed to formation of denser structure (Swioklo et. al, 2016). The mass transfer through the hydrogels is also related to the alginate concentration which can be used to control nutrient flow from hydrogels (Swioklo et. al, 2016). The denser gel network offered by higher alginate concentrations may also offer protection against ACN diffusion out of the hydrogels before the target is reached. KC alone gels at lower temperatures, but above 40 ◦C has a weaker gel strength.

The molecular structures of the three commonly used carrageenan, iota, kappa, and lambda are displayed in Figure 9. Each of the three are sulfated polysaccharides composed principally of alpha-D-galactopyranose-4-sulfate units and 3,6-anhydro-alpha-D-galactopyranose units (Towle, 1974), differences between the three being their number of sulfate groups. Higher levels of sulfated polysaccharides lead to lower solubility temperatures and lower gel strength in the iota and lambda carrageenan, making them less suitable than kappa carrageen for hydrogel formation (Porto, 2003).

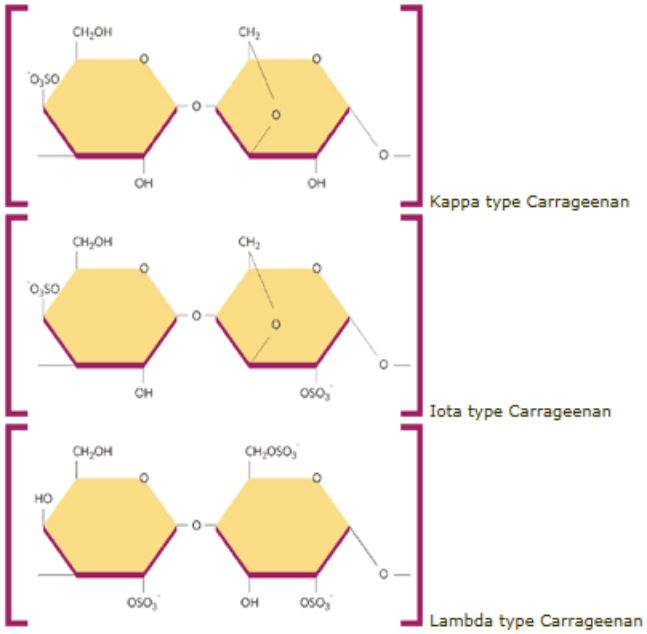

Figure 9: Carrageenan structures (Porto, 2003)

KC is known to be reactive with potassium ion and useful in the food industry for gel formations, it does however only form rigid and brittle hydrogels (Porto, 2003) so the strengthening properties of alginate are very beneficial for hydrogel formation. KC is also subject to syneresis, the contraction of the gel with the separating of the liquid, which is another driving factor for mixing it with alginate before gel formation. Other common mixed hydrogel option for the kappa is iota carrageen; which may reduce diffusion of contents but increase brittleness of the gel, or

locust bean gum; which may reduce the amount of kappa needed but be very costly (McHugh, 2003).

Our experiments involved heating the carrageenan and alginate to 70 ◦C in order to dissolve them in water and then cooling the solution to 40 °C for gel (Towle, 1974). According to literature, the dissolving over heat and cooling are separate steps before the exposure to potassium salt because of kC's solubility in potassium ions. Our experiments accounted for this solubility by keeping the gel warm enough to still flow through the pump for hydrogel creation, but allowing it to cool enough to form a gel before dripped into a $CaCl_2$ + KCl solution.

In dripping the gel solution into the $CaCl_2$ + KCl containing curing solution, our experiments formed hydrogels similar to those reported in literature. The gel droplets formed a hardened outer shell, characteristic of hydrogels, once dropped into the solution of divalent cations, calcium in our case (Lakkis, 2016). KC is known as being capable of forming gels at room temperature, but to insure a stable temperature environment for our hydrogels we placed them into a refrigerator during the curing process.

The analysis of moisture content of the particles after desorption in the desiccators by using various salts showed that at low relative humidity values, lower than 33%, higher moisture contents than expected values were observed. One hypothesis for the cause of such high moisture contents could be that at low relative humidities moisture removal from the surface of the particles are faster than the moisture migration to the surface resulting in the particle surface drying and forming an impermeable shell around the particle and trapping the moisture inside. To prevent the hydrogels from drying too quickly on the outside surface it might be necessary to start the desorption process at higher relative humidity and move the hydrogels progressively to lower relative humidity environments.

In the encapsulation efficiency determinations, the second and third purple corn trials showed similar encapsulation efficiencies, but much lower (~10%) than that of the first purple corn trial. One possible explanation for this is the difference in the concentrations of the starting ACN solutions, which can be seen in the data table A3 in the appendix. The initial concentration of ACN for the first purple corn trial was 160 µg/mL while for the second trials was 111 µg/mL. The encapsulation efficiencies were very similar of the two blueberry ACN concentrations, which again may be due to the similarity of the initial ACN concentrations, which differed by less than 0.001 µg/mL. There were also noticeable differences between the blueberry containing hydrogel encapsulation efficiencies and those of the purple corn hydrogels. The two blueberry trials although had lower initial ACN concentrations than those for purple corn hydrogels, they had higher encapsulation efficiencies. These differences are believed to be caused by the botanical differences between the blueberries and purple corn and the chemical structures of the ACN extract from them.

Oven drying parameters to obtain stable hydrogel particles were investigated. Typically, oven drying is used to reach a complete dehydration of hydrogels made from alginate alone. These tests also showed lower final moisture contents then air drying the same particles. A limitation of oven drying is that the exposure to high temperatures may cause the hydrogels to become brittle and crack along the surfaces, amongst the pores, which would lead to hydrogel weakness upon rehydration (Das, 2008). This was also observed in our laboratory when alginate and pectin hydrogels which were dried completely were rehydrated. The rehydrated hydrogels could not maintain their integrity and broke into pieces. The formation of cracks on the surfaces would lead to a more rapid release of the encapsulated material, which would mean a less effective delivery of the ACNs.

The initial goal of the oven drying was just to stabilize the product by lowering the moisture content to below 0.4. In the evaluation of ACN losses from the hydrogel when they were placed within a solution simulating liquid beverage, it was also observed that the dried hydrogels showed less ACN diffusion; which is an additional benefit of drying the hydrogels. Drying the hydrogels proved to be very beneficial in the diffusion test conducted because the dry hydrogels had a much lower equilibrium ACN value than the wet ones. This may be due to the pores on the hydrogel surfaces closing during the drying process, which allow for slower ACN diffusion upon rehydration.

Conclusion

There were large encapsulation efficiency differences observed between the purple corn ACN particles with different initial ACN concentrations. Encapsulation efficiency increased with increasing initial ACN concentration within the range studied (111-161µg/mL).

Higher encapsulation efficiencies for blueberry ACN hydrogel particles than purple corn ACN particles were obtained although initial ACN concentration for blueberry was 10 times smaller than for purple corn. The differences in the encapsulation efficiencies between purple corn and blueberry hydrogels are believed to be caused by differences in the chemical structures of the ACN from different botanical origin.

The moisture content of the particles after drying in the desiccators with the various salts, of various relative humidity, were higher than expected values. The hydrogels placed into the lowest of the relative humidity environments may require exposure to first higher relative humidity environments and then progressively lower environments.

Oven drying trials conducted thus far indicated that a lower activity value of 0.4 can be achieved after 2 hours of drying. At 0.4 water activity, the hydrogels would be at the shelf stable but not dried so far that they are brittle and disintegrate upon rehydration.

Oven drying also decreased the ACN loss from hydrogels upon rehydration.

Recommendations and Suggestions

The initial ACN concentrations between the purple corn trials are believed to be the cause of the encapsulation efficiency. The hypothesis can be tested by designing future trials with varying initial ACN concentrations to find the optimum initial concentration.

The blueberry ACN tests should also be repeated changing initial concentration of blueberry ACN to evaluate its effect on the encapsulation efficiencies, and so that diffusion studies may be conducted and compared with those from the purple corn trials.

The experiments conducted in this study involved leaving the desiccators in the open, where they were exposed to no sunlight but they were exposed to light when the lights in the room were turned on. For future desiccator studies the desiccators should be placed into a dark cabinet or room so the hydrogels are not exposed to the light. ACN is known to be unstable in light so to measure the effects of drying, and drying alone on ACN diffusion it may necessary to dry the particles in the dark.

Desorption studies using desiccator should be redesigned to evaluate the effect of successive exposure from high to low relative humidity values on moisture removal and equilibrium moisture content of the hydrogels.

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Appendix

TABLE A1: The three purple corn trials equilibration moisture content data

Salt	**a_w**	Trial 1 Moisture Content Fraction After Equilibration		Trial 2 Moisture Content Fraction After Equilibration		Trial 3 Moisture Content Fraction After Equilibration	Average Moisture Content Fraction After Equilibration
KOH	0.0824	0.48499	0.46598	0.49607	0.49431	0.49635	0.420017
LiCl	0.11	0.48244	0.47249	0.49946	0.51217	0.49905	0.429268
KCH_3COO-	0.23	0.50498	0.50366	0.49516	0.51283	0.52503	0.461943
MgCl	0.33	0.56565	0.57354	0.58728	0.58628	0.59668	0.539905
K_2CO_3	0.43	0.5974	0.60238	0.63608	0.63036		0.579244
$Mg(NO_3)_2$	0.529	0.65745	0.65372	0.67625	0.68806		0.640896
NaCl	0.75	0.67759	0.67624	0.67319	0.67178		0.689760
KCl	0.85	0.72998	0.72973	0.74702	0.73543		0.758432
KNO_3	0.94	0.83613	0.8352	0.89134	0.88842		0.878218
K_2SO_4	0.973	0.96584	0.96569	0.96369	0.96349		0.966342

TABLE A2: The two blueberry trials equilibration moisture content data

Salt	**a_w**	Trial 1 Moisture Content Fraction After Equilibration		Trial 2 Moisture Content Fraction After Equilibration		Average Moisture Content Fraction After Equilibration
KOH	0.0824	0.46881	0.47511	0.4733	0.46975	0.47173
LiCl	0.11	0.45819	0.46255	0.4663	0.4976	0.47115
KCH_3COO-	0.23	0.5009	0.51357	0.4845	0.49293	0.49797
MgCl	0.33	0.55761	0.54883	0.5804	0.58456	0.56785
K_2CO_3	0.43	0.60524	0.59678	0.624	0.62737	0.61336
$Mg(NO_3)_2$	0.529	0.6497	0.64374	0.6694	0.65895	0.65544
NaCl	0.75	0.65006	0.65281	0.6707	0.66803	0.66041
KCl	0.85	0.7083	0.71013	0.7163	0.7084	0.71078
KNO_3	0.94	0.90601	0.90678	0.8375	0.83903	0.87232
K_2SO_4	0.973	0.9645	0.9639	0.9612	0.95956	0.96229

TABLE A3: Calculation table for encapsulation efficiencies

	Purple Corn Trial 1 Data	Purple Corn Trial 2 Data	Purple Corn Trial 3 Data	Blueberry Trial 1 Data	Blueberry Trial 2 Data
Curing Study					
Flow rate of solution	13.00	13.00	13.00	13.00	13.00
PW/SV in curing bath and diffusion experiment	0.14	0.14	0.14	0.14	0.14
Initial Anthocyanin bulk solution (µg/ml)	2054.82	1425.85	1426.72	118.56	118.56
Wt of solution before curing (g)	13.30	13.65	13.33	14.58	16.63
Wt of particles (g) after curing (g)	10.34	9.90	9.83	10.59	12.20
Moisture content in particles (initial)	0.98	0.98	0.98	0.98	0.98
Moisture content in particles (final)	0.97	0.97	0.97	0.97	0.97
Anthocyanin concentration in gel solution (µg/ml)	160.98	111.70	111.77	9.29	9.29
Initial Anthocyanin amount in the dropped gel solution (µg)	2140.99	1524.74	1489.91	135.42	154.46
Concentration of Anthocyanin in particle liquid phase (µg/ml)	164.33	113.98	114.05	9.48	9.48
Curing bath volume initial (ml)	95.59	98.20	98.01	104.58	119.64
Curing bath volume final (ml)	98.55	101.95	101.51	108.57	124.07
Abs of curing bath after curing with dilution	0.49	0.39	0.38	0.02	0.02
Dilution factor	2.00	2.00	2.00	2.00	2.00
Concentration of anthocyanin in curing bath (µg/ml)	16.67	13.07	12.75	0.79	0.79
Anthocyanin amount in curing bath after curing (µg)	1642.48	1332.21	1293.88	85.40	98.11
Anthocyanin amount in particles after curing (µg)	498.50	192.53	196.03	50.02	56.35
Encapsulation efficiency (%)	23.28	12.63	13.16	36.94	36.48